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Review

Suppression of epithelial ovarian cancer invasion into the omentum by 1α,25-dihydroxyvitamin D_3 and its receptor

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ABSTRACT

Epithelial ovarian cancer (EOC) is the leading cause of gynecological cancer death in women, mainly because it has spread to intraperitoneal tissues such as the omentum in the peritoneal cavity by the time of diagnosis. In the present study, we established *in vitro* assays, *ex vivo* omental organ culture system and syngeneic animal tumor models using wild type (WT) and vitamin D receptor (VDR) null mice to investigate the effects of 1α,25-dihydroxyvitamin D_3 ($1,25D_3$) and VDR on EOC invasion. Treatment of human EOC cells with $1,25D_3$ suppressed their migration and invasion in monolayer scratch and transwell assays and ability to colonize the omentum in the *ex vivo* system, supporting a role for epithelial VDR in interfering with EOC invasion. Furthermore, VDR knockdown in OVCAR3 cells increased their ability to colonize the omentum in the *ex vivo* system in the absence of $1,25D_3$, showing a potential ligand-independent suppression of EOC invasion by epithelial VDR. In syngeneic models, ID8 tumors exhibited an increased ability to colonize omenta of VDR null over that of WT mice; pre-treatment of WT, not VDR null, mice with EB1089 reduced ID8 colonization, revealing a role for stromal VDR in suppressing EOC invasion. These studies are the first to demonstrate a role for epithelial and stromal VDR in mediating the activity of $1,25D_3$ as well as a $1,25D_3$-independent action of the VDR in suppressing EOC invasion. The data suggest that VDR-based drug discovery may lead to the development of new intervention strategies to improve the survival of patients with EOC at advanced stages.

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Contents

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1. Introduction

EOC is the deadliest among all gynecologic cancers and causes more deaths than cervical and uterine cancers combined. Despite the overall advancement in cancer research and clinical development over recent decades, there has been little improvement in EOC mortality rates. The poor prognosis is mainly due to the disease reaching an advanced stage before it is discovered. Primary cytoreductive surgery followed by chemotherapy with cisplatin and paclitaxel is the standard treatment regimen for patients with advanced EOC, yielding high response rates and improving both short and medium-term survivals. However, most patients will eventually relapse and die of their cancer [32]. Intervention strategies that suppress EOC invasion would retain the cancer inside ovaries, allowing simple surgery to suffice as a cure.

Omentum is a sheet-like tissue attached to the greater curvature of the stomach. It is the most common site for EOC metastatic spread [19,20,36]. The spread happens rather quickly and, in 80% of EOC patients, the cancer usually has spread to this tissue at the time of diagnosis. The composition and function of omenta are similar between mice and human. They contain primarily adipose tissue and islands of compact immune cells called milky spots, which is a lymphoid organ controlling peritoneal cavity immune response [33]. A recent study has reported that omental adipocytes promote EOC metastasis by providing energy for rapid tumor growth [30]. Nevertheless, the mechanisms underlying EOC invasion into the omentum are largely unknown and key molecular events controlling the process remain to be defined.

$1,25D_3$ is a fat-soluble seco-steroid hormone best known for its role in calcium and phosphate homeostasis. Effects of $1,25D_3$ are mediated through the VDR that belongs to the steroid/thyroid hormone receptor superfamily [9,39]. In addition, $1,25D_3$ and its analogs elicit anti-tumor effects in a wide variety of cancer cell types through the induction of cell death, cell cycle arrest, differentiation, angiogenesis, etc. [3,6,7,11,12,37,38,40,41,45], suggesting that $1,25D_3$ holds great promise in cancer intervention. In EOC cells, $1,25D_3$ causes cell cycle arrest at the G2/M transition through p53-independent induction of GADD45 [16]. Further studies have identified p27 as the key mediator of $1,25D_3$-induced growth arrest in G1/S checkpoint [23] and defined a decrease in hTERT mRNA stability through microRNA as the mechanism underlying $1,25D_3$-induced cell death [15,17]. However, a role for $1,25D_3$ and VDR in EOC invasion and metastasis has not been investigated.

In the present studies, a series of experiments employing *in vitro*,*ex vivo* and *in vivo* EOC tumor models were conducted to assess the possible involvement of $1,25D_3$ and VDR in suppressing EOC invasion into the omentum. These studies have revealed a novel role for $1,25D_3$ in suppressing EOC invasion through both epithelial and stromal VDR. The findings suggest that VDR-based drug discovery may lead to a new intervention strategy to improve the clinical outcomes of patients with advanced EOC.

2. Materials and methods

2.1. Cell culture and reagents

OVCAR3 human ovarian carcinoma cells (American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium supplemented with 15% calf serum (CS), 2 mM L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/l glucose, 1.5 g/l sodium bicarbonate and 10 μg/ml bovine insulin. SKOV3-Luc cells, human ovarian carcinoma cell line, (Cell Biolabs, San Diego, CA) were maintained in DMEM containing 584 mg/l L-glutamine and 4.5 g/l glucose, supplemented with 5% CS, 100 units/ml penicillin, 100 μg/ml streptomycin and 500 μg/ml geneticin. ID8-VEGF murine ovarian cancer cells have been described elsewhere in detail [42]. The cells were generated by transfecting ID8 cells with a retroviral vector containing green fluorescent protein (GFP) and VEGF164, which accelerated tumor growth and ascites formation, significantly enhanced tumor angiogenesis, and substantially promoted the survival of tumor cells *in vivo* [35]. Cells were maintained in DMEM supplemented with 5% CS, 100 units/ml penicillin, and 100 μg/ml streptomycin.

$1,25D_3$ (calcitriol) was purchased from Calbiochem (La Jolla, CA). EB1089 (seocalcitol) was generously provided by Leo Pharmaceutical Products (Ballerup, Denmark). They were reconstituted in 100% ethanol (EtOH) and stored protected from light at −20 °C. All handling of $1,25D_3$ and EB1089 was performed with indirect lighting.

2.2. Stable transfections with luciferase and VDR short hairpin RNA (shRNA)

To establish cells stably expressing luciferase, OVCAR3 and ID8-VEGF cells were transfected with 1 μg of pGL3-control plasmid (Promega, Madison, WI) using Lipofectamine 2000 (Invitrogen, Grand Island, NY) following the protocol from Invitrogen. Stable transfectants were established after selection in medium containing 400 μg/ml (for OVCAR3-Luc) or 800 μg/ml (for ID8-VEGF-Luc) G418 for a period of about 4 weeks.

For the establishment of OVCAR3 cells stably expressing control or VDR shRNA, cells were transfected with 2 μg of control pFIV-H1-Puro vector or shVDR [26] using the Lipofectamine 2000 in 2 ml of Opti-MEM medium (Invitrogen, Grand Island, NY). 4 h post transfections, the cell were re-plated in RPMI medium containing 10% CS and 2 μg/ml puromycin for 48 h. Cells were then split and placed at low density. Stable clones were achieved through selection with 2 μg/ml